In Vitro Assessment of Biodurability: Acellular Systems

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The assessment of biodurability of man-made vitreous fibers is essential to the limitation of health hazards associated with human exposure to environments in which respirable fibers are present. *In vitro* acellular systems provide effective test methods of measuring fiber solubility provided care is taken to select the most suitable solvent and test conditions for the specific fiber type and dimension. — Environ Health Perspect 102(Suppl 5):47–53 (1994)

Key words: man-made vitreous fibers, MMVF, *in vitro*, acellular, biodurability, durability

Introduction

As soon as it was recognized that certain natural mineral fibers could represent a health hazard if inhaled, research started on the possible health effects of other particles that were essentially artificial, mineral or organic fibers. Studies on animals and humans showed very quickly that certain artificial mineral fibers, such as insulation wool fibers, produced effects that were not identical to those produced by natural mineral fibers.

There are two main reasons for the difference between man-made mineral fibers and natural fibers. First, a totally different level of exposure exists for man-made mineral fibers (1 fiber/ml) than existed for natural fibers (10,000 fibers/ml), and second, fiber geometry is very different (*1*), for asbestos fibers can have diameters as small as 0.2 µm, due to splitting, compared with an average diameter for man-made mineral fibers of approximately 3 µm (*2*).

It is also generally recognized that fiber biosolubility or biodurability is a very important parameter that influences the possible health hazard caused by inhalation of fibers. To present a health risk, a fiber must be not only thin and long enough, but also durable (*3*). This difference in durability was first demonstrated in a very simple experiment: fibers were placed in a static system that simulated human extracellular fluid or the fluid of the lung (*4*). Later, the system was improved by the introduction of a dynamic system in which fluid flowed around the fibers (*5,6*).

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Test Parameters

Some of the many methods that have been published, both in scientific journals and in patents on biosoluble fibers (*7–12*), are analyzed in Table 1. However, no real standardization of the methodology has yet appeared, and the main differences among all these methods are in the type of fluid used; the morphology of the test fibers; and the type of test, whether static or dynamic. In only one study have measurements with polymeric organic fibers been reported (*13*).

Types of Fluid

"Extracellular" Fluids. Most of the studies have used Gamble's solution, or an adaptation of this type of fluid (*14,15*). However, slight variations in fluid composition do not seem to be responsible for important differences in some of the solubility data; this is one parameter on which there is general agreement and where standardization should be the easiest (*16*). Currently used fluid compositions simulating the extracellular milieu, with pH buffered at 7.4 to 7.6, are given in Table 2. The cations present and the buffers in the solutions may influence the formation of chemical complexes with the leached glass.

Other Fluids. Among the 17 studies analyzed (Table 1), only 5 mention that instead of using the extracellular fluid with pH 7.5, fluid simulating intracellular conditions with pH at 4.5 to 5.0 may be used (*17–21*). This is intended to simulate the pH encountered when fibers are captured by macrophages. Solubility tests have shown that fibers soluble at pH 7.5, simulating extracellular fluid, are far less soluble at pH 4.5, simulating intracellular conditions. The reverse is also true. It is possible to imagine glass compositions that, while being reasonably soluble at pH 7.5, are still adequately soluble at pH 4.5 (*20*). However, it has not yet been shown that fiber solubility in intracellular fluid simulant is relevant to animal experiments, since it has not been shown that fibers that are highly soluble at pH 4.5 and either insoluble or less soluble at pH 7.5 do not produce fibrosis or tumors in experimental animals.

Morphology of the Fibers

In most studies the fibers used were obtained by sampling commercial fibers, with a mean diameter approximately 3 µm and a variable range distribution. Fiber lengths often are reduced by the sampling procedure.

Fibers of different chemical compositions have been produced under laboratory conditions by a drawing process that gives a diameter of approximately 10 µm, and this type of fiber has been used in solubility studies (*15,17*).

Fibers with very different diameters but with the same chemical composition may appear to have different solubilities; but when the mass of fibers is adjusted to give approximately the same surface area, the solubility difference almost disappears. Alternatively, by calculating the dissolution coefficient, expressed as nanometers/day (nm/day), the effect of fiber diameter is eliminated (*5*).

Theoretically, from the standpoint of the associated health hazard, the morphology (i.e., mainly the diameter) of a fiber may not be as important as its specific

Table 1. Methods to measure solubility.

Reference		Fluid	pH	Fiber sample	Test system	Measurements	No. fibers measured	Results
Tiesler, 1981	(*30*)	Water (without buffer)	7.5 (→9 during attack)	Textile glass (E) Glass-, rock-, slagwool (all of commercial grade) D: #3 µm	Quasi continuous	Dissoluted components	13	Durability decrease: textile>rockwool> slagwool>glasswool Influence of pH change during attack caused by leached alkalis
Leineweber, 1982	(*24*)	Derived from Gamble	7.5 ↑ 9.0	6 siliceous D: #3 µm	Continuous 1440 ml	Remaining weight ng/cm²/hr	6	10–300 ng/cm²/hr
Förster, 1982	(*31*)	Derived from Gamble	7.5 ↑ 8.9	Chrysotile Crocidolite 20 MMMF (all of commercial grade) D: 0.02–5 µm	Stationary, shaking	Si in solution Si loss ng/cm²/hr	22	10 ng/cm²/hr
Klingholz and Steinkopf, 1982	(*32*)	Derived from Gamble	7.5	Glass-, rock-, basalt-, slag wool	Stationary continuous	Si in solution ↑ Si loss and other components	4	Leaching of the different components
Förster and Klingholz, 1982	(*33*)	Derived from Gamble	7.4 ↑ 9.0	6 MMMF D: 3 µm (all of commercial grade) D: 3 µm	Static	Si loss ng/cm²/hr and µm/year	6	10–100 ng/cm²/hr
Feck, 1984	(*25*)	Derived from Gamble	8.2 ↑ 9.3	19 MMMF 4 natural (all of commercial grade) D: 3 µm	Static, dynamic	Important components in solution	23	4–170 ng/cm²/hr 0.14–6.0 µm/year
Scholze and Condradt, 1987	(*34*)	Derived from Gamble	7.6 ± 0.2	7 vitreous 3 refractory 3 natural D: 0.005–4.9 µm	Dynamic (40 ml/day) 200 ml/g day	Si in solution Si loss (B, K) nm/day and µm/year	13	0.08–1.3 µm/year
Bauer and Law, 1988	(*26*)	Gamble, modified	7.6 ± 0.2	Glasswool D: <3 µm	Dynamic 120 ml/day	Si in solution % SiO_2/day extracted in 6 months	10	0.000021–0.30% Si/day
Larsen, 1989	(*35*)	Gamble, modified	7.5	Glass-, rockwool Glass microfibers Natural mineral fibers	Static	Initial dissolution rate (from Si loss)	6	5–860 ng/cm²/day
Rockwool patent, 1990	(*10*)	Derived from Gamble		Mineral fibers D: 3µm	Static 5 hr	Si in solution ppm SiO_2 loss	7	1.84–10.80 ppm SiO_2
Potter and Mattson, 1991	(*15*)	Kanapilly and Gamble, modified	7.4 ± 0.2	30 glasses D: 10 µm, 2 µm	Dynamic 290 ml/day	Weight loss components in solution ppm SiO_2 loss	30	0.9–887 ng/cm²/hr
Thélohan, 1992	(*17*)	Derived from Gamble	7.4 or 4.5	D: textile 10 µm Special: 0,5,3,20 µm Standard: 3 µm 62 compositions: glass and rock	Dynamic Identical to Scholze and Condradt Variable flow rate	Si in solution loss Si in % nm/day	165	Influence of · Sample mass · Fiber diameter · Flow rate
Baillif and Touray, 1992	(*23*)	Kanapilly and Gamble, modified	8.0 ↓ 8.8	4 glasses	Static 30 mg in 250 ml Dynamic 50 mg in 40 ml/day	Si in solution weight loss ng/cm²/hr	4	Influence of Al and P content 115–6580 ng/cm²/hr
Baymel, 1992	(*21*)	Kanapilly, simulated	7.6	Sized microspheres 8 glasses	Dynamic 288 ml/day 29 ml/day	Si loss ng/cm²/hr	8	
Sebastien, 1992	(*18,19*)	Derived from Gamble	7.5, 5.0	Glasswool and slagwool fibers	Dynamic 40 ml/min 40 days	Si loss ng/cm²/hr and nm/day	78	Influence of chemical composition, pH of fluid and manufacturing process 0.94–509 ng/cm²/hr
Hesterberg, 1992	(*21*)	Gamble	7.4, 4.0	Glasswool, slagwool	Dynamic 40 ml/day	Si loss ng/cm²/hr	5	Soluble fibers pH 4.5, 4.7
Christensen, 1992	(*21*)	Gamble, modified	7.5 , 4.7	Glasswool, rockwool, slagwool	Static 4 days	Si in liquid	5	Rockwool more soluble at pH 4.5 than at pH 7.5

Abbreviations: MMMF, man-made mineral fibers.

Table 2. Simulated lung fluid solutions[a].

Lung fluid solutions	Modified from Kanapilly et al. (*14*)	Modified from Scholze and Condradt (*5*)	Modified human blood plasma
Ca^{2+}	7.9	69.6	95
Na^{+}	3363.3	3516.8	4267
K^{+}	—	—	166
Mg^{2+}	—	21.3	25
NH_4^{+}	180.4	—	1
Cl^{-}	4481.4	4085.0	3651
HCO_3^{-}	1647.3	1963.3	1216
PO_4^{3-}	114.2	87.9	287
SO_4	48.0	53.4	32
Citrate	37.9	119.6	3171
Glycine	450.0	118.0	—
Tartrate	—	115.8	—
Lactate	—	139.1	—
Pyruvate	—	136.1	—
Dextrin	—	—	3600
N_3^{-}	64.6		
Formaldehyde	[2000]		
Methanol	[655]		
Isothiazolin	{0.150}		

[a] Entries in mg/l.

chemical composition. Nevertheless, the production process may also be important (*22*), since manufacturing fibers of different diameters uses different production processes. It may be that it is not the change in diameter that changes the durability, but changes in the production process itself. In summary, therefore, to compare the durabilities of different fibers, they should be produced by the same manufacturing process and fall within the same diameter range.

Since it is important to compare the results of *in vitro* tests with *in vivo* animal experiments, in which, for the most part, Thermal Insulation Manufacturers Association (TIMA) fibers are used, it would be prudent to use this type of fiber in *in vitro* tests also. Essentially, 80% of fibers should be <1 μm in diameter, and half should be 5 to 15 μm in length.

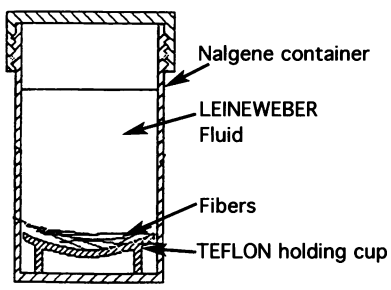

Figure 1. Static method. Either 30 fibers are treated in 250 ml of solution, or 30-mg fibers in 30 ml of solution (*17*).

Static versus Dynamic Tests

The early studies used mainly static tests (Figure 1), but dynamic tests are now more frequently used (Figure 2). The continuous-flow model is based on the theory that *in vivo* the single fiber is attacked by a large volume of intracellular (or extracellular) fluid of which the pH, osmotic pressure and other parameters are stable, but in which the concentration of dissolved glass constituents is very low. The continuous flow model may, therefore, better simulate *in vivo* conditions (*15,21*).

For a very limited number of fiber compositions, it has been shown that solubilities measured in a static or a dynamic test are very closely correlated (*23*); and therefore the static test, which is easier to control and has smaller internal variation, could be used, provided each test is repeated.

In the dynamic test, one of the important parameters is the flow rate, which initially was proposed at 40 ml/day (*5*). Subsequently, variable flow rates have been used (*15*), depending on the solubility of the fibers to be tested. The results of solubility tests will, in fact, depend on two major parameters, the volume of the solution or flow rate (Figure 3) and the mass of the fibers or their surface area.

The current tendency is to use a variable flow rate to maintain the kinetic parameter s/v constant (where s = surface area of the sample and v = the flow rate), and the flow rates should be chosen, so that there is as good a correlation as possible with animal experiments, as indicated in Figure 4 (*21*).

The difficulties involved should not be underestimated. On the one hand, a good flow rate must be chosen and then controlled; on the other, the diameters of the test fibers must be measured with sufficient precision, if the calculation of the surface area is to be reasonably accurate. This can

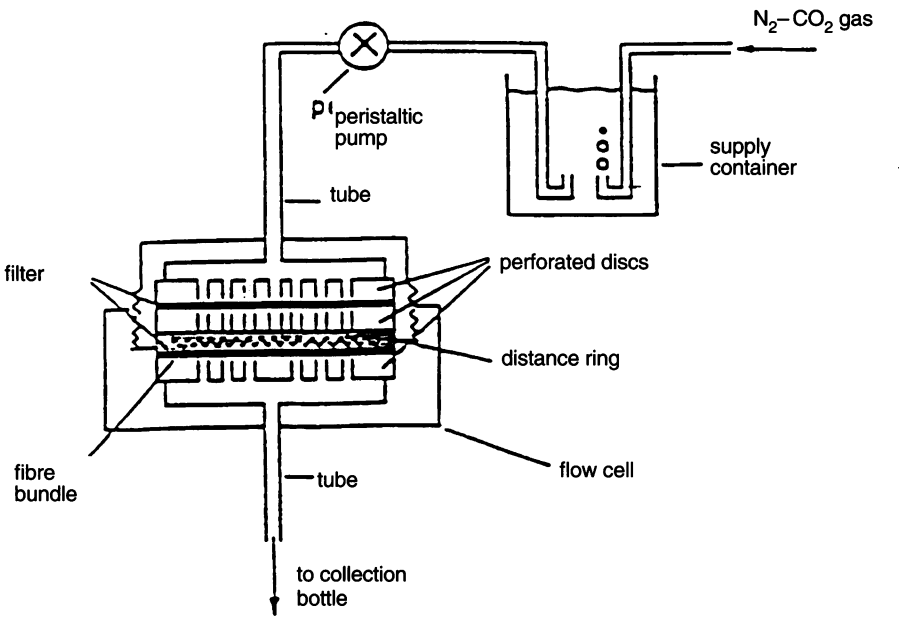

Figure 2. Dynamic method. 200 mg of fibers treated by solution buffered at pH 7.4 to 7.5 at a flow rate of 40 ml/day (*5*).

be best achieved with fine fibers by using a BET measurement.

Methods of Expressing the Results

What Is Measured?

Having set out the practical variables in *in vitro* experiments—solution composition and pH, dimensions of the fiber samples used, rate of flow-through of the solution—consideration must be given to the different types of measurements that are made.

Loss of Silica. In most studies, the loss of silica from the test fibers and the consequent concentration of silica in the solution have been measured. The validity of this approach depends on silica being a major component of MMVF and an essential part of the glass matrix (*5*). Moreover, the measurement of silica in the solution is easy, and, in congruent dissolution, gives a valid answer.

Weight Loss. Measurement of weight loss from the test sample has been used (*24*), but this is probably not very accurate because of the limited mass of the samples. The weight loss measurement method also presents other difficulties, since the leached layers of the fibers may absorb some components from the fluid, including calcium, phosphate, and water.

Total Loss of Ions. In more recent studies of dissolution, measurements have been made of the major components in the effluent solution (*15,25*). This approach is more costly because many measurements have to be made; but it is also more accurate and should be used especially for fibers in which silica is not the major component or for which dissolution is markedly noncongruent, as, for example, is the case for some slagwool fibers. With this method it is possible to make comparison of durability over a wide spectrum of compositions. In addition, it gives both dissolution and leaching rates, and is, therefore, an approach to be recommended for future studies.

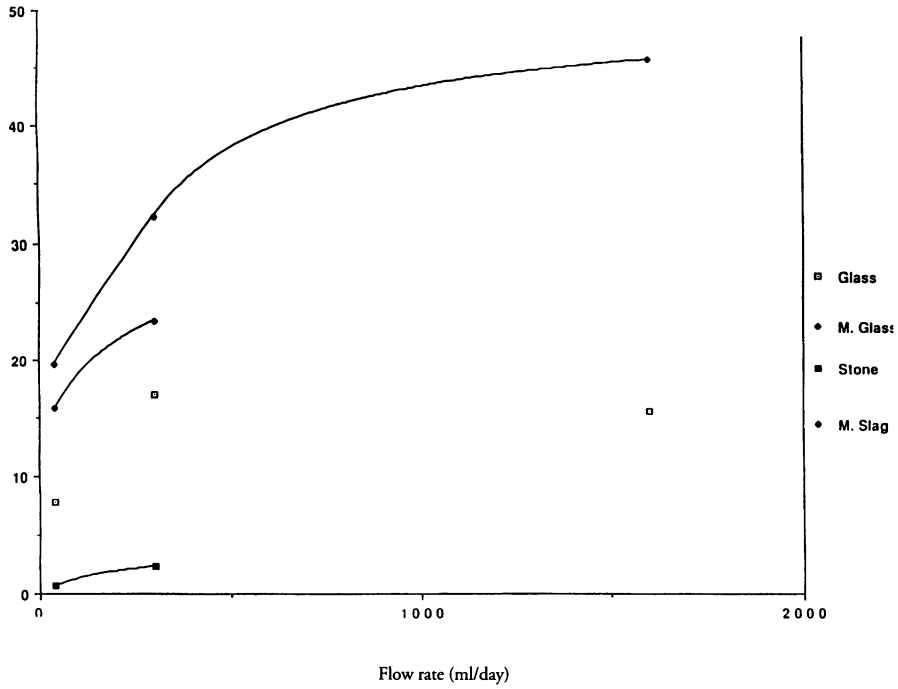

Figure 3. Influence of the flow rate on the dissolution of different fibers (same geometry) (*17*).

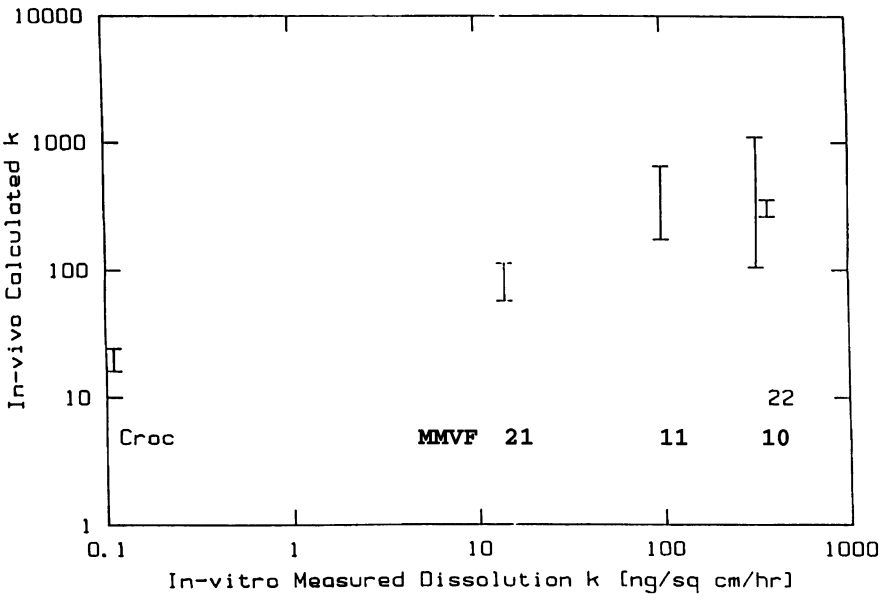

Figure 4. *In vivo* versus *in vitro* dissolution rate (*21*).

How Are the Measurements Expressed?

Alternative Expressions. Whatever the type of measurement, whether it be loss of silica or other ions or total weight loss, the results are reported as a function of time. To express the solubility by one coefficient, the decrease in diameter with time—expressed as nm/day—has been proposed (*5*). This method assumes that the fiber diameter distribution is known. Clearly, a complex calculation would be necessary to derive the coefficient, nm/day, from the results based on loss of silica or other ions. An alternative expression has been proposed based on weight loss per surface area versus time, ng/cm^2/hr. (*15*). The two expressions are mathematically related. If the density of the glass is known and assumed to remain constant, it is easy to convert nm/day to ng/cm^2/hr. However, both methods have the disadvantage of being based on assumptions regarding the distribution of fiber diameters. Nonetheless, the two expressions are widely used; but it would be better if we could find a way to represent results that would be easier to manipulate and were based on fewer assumptions. The percentage of mass extracted after 6 months has been proposed as an alternative (*26*).

Dissolution Rates. If results are expressed as a dissolution rate constant, k, for each glass composition, one unique value for k easily can be computed irrespective of the manner in which the test is run, provided the glass is of low solubility or has a totally congruent dissolution. But for high solubility glasses or for glasses with noncongruent dissolution, several values of k can be computed :

- *an initial k*, which tends to be high and represents the rapid leaching of one element, such as calcium, from slagwool (*21*);
- *an ultimate k*, which tends to be low and represents the leaching of the modified fiber after a given time; or
- *a mean k*, computed as a running average of the two previous values; this k has often been reported in published results (*3,17,18*).

As with the choice of the flow rate, the choice of the dissolution rate constant, k, should be such as to provide the best correlation possible with animal experiments.

Results

Despite the wide range of parameters that have been used in the different methods, a certain number of conclusions can be drawn from the available results. A fiber composition with a high aluminium content will have a low solubility, at least at pH 7.4. An increase in the concentration of alkaline oxides or alkaline-earth oxides in the composition of a fiber will increase the solubility at pH 7.4; but because the compositions of glass fibers are complex, the relationship between the solubility and the chemical composition of a fiber is not linear. Moreover, even given identical chemical compositions, the solubility of fibers can be influenced by their production method and probably also by the aspect of the fiber surface.

Validation of Results

The comparison between *in vitro* assessment of durability and the results of *in vivo* experiments is made very difficult because most measurements *in vitro* are reported as a mass loss or its equivalent, whereas most *in vivo* experiments give results as fiber counts. One document that attempts to relate these quantities was published recently (*27*), and it appears that the discrepancies in reporting the durabilities in different ways may not be so complete as was thought at first.

Results of *in vivo* studies often are interpreted as obeying first order kinetics. For example, when the elimination of

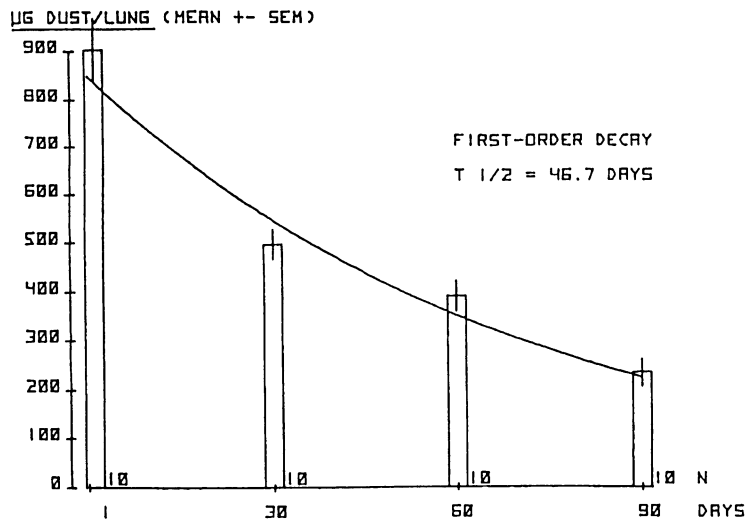

Figure 5. Decrease of lung dust in rats following inhalation (*2*).

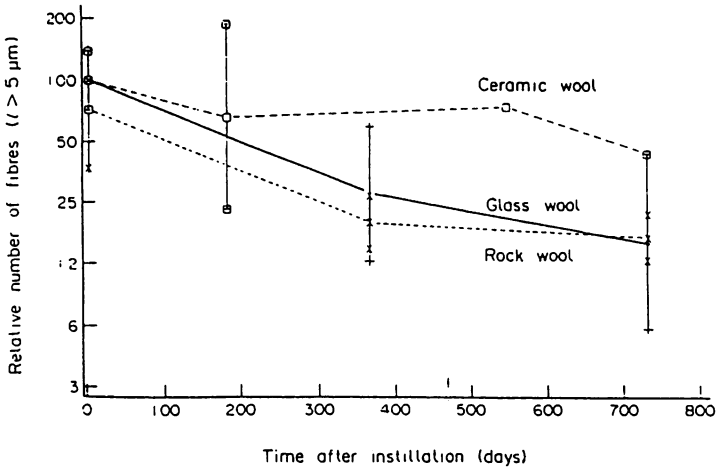

Figure 6. Relative number of mineral wool fibers (length >5 µm) in the lung ash recovered from rats at different sacrifice dates (*28*).

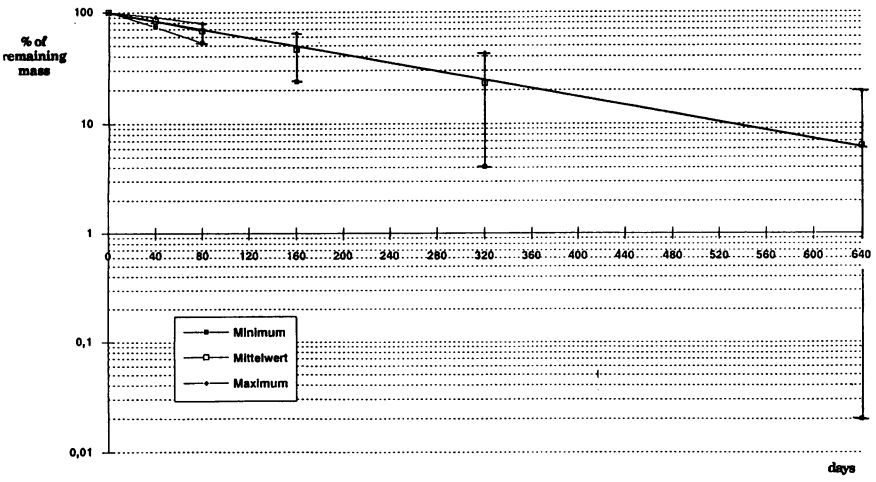

Figure 7. Decrease of mass of fibers with time (*30*).

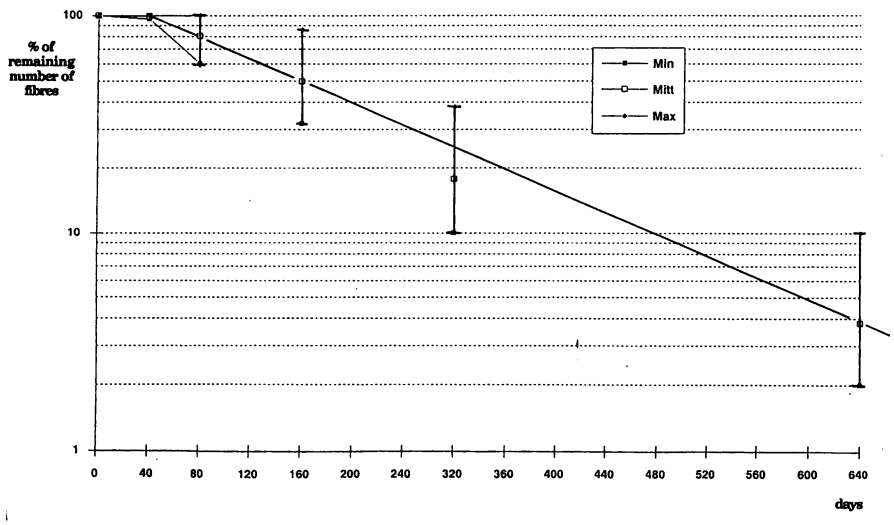

Figure 8. Decrease of number of fibers with time (*30*).

inhaled glasswool dust from rat lungs is expressed by the mass of dust remaining per rat lung and plotted against time after exposure, the results appear to be consistent with a first-order reaction law (*2*) (Figure 5).

Similarly, the results of an intratracheal study, based on the decrease of fiber number in rat lung ash, also appeared to obey first order kinetics (*28*) (Figure 6). However, taking into consideration the limitations in the accuracy of fiber number determination, and the fact that the results were based on only three measured points, all the three curves could just as well be straight lines (by linear regression), lying within the field of standard deviation of each measured point.

By using the equation by which *in vitro* test results are calculated (*15*), it has been possible to calculate, step by step, for any given duration, the mass loss or the decrease in diameter of any fiber from an original sample (*29*). Similarly, it has been possible to calculate and simulate the number of remaining fibers (Figures 7, 8).

These curves are very similar to those found in *in vivo* studies (*2,28*), where what was measured was the number of fibers remaining. It appears, therefore, that the published *in vivo* and *in vitro* results are not contradictory, and that *in vitro* experiments give very valuable information on fiber dissolution durability while *in vivo* experiments may also give information on fiber clearance.

Conclusion

The ultimate aim of studies involving different methods of measuring durability *in vitro* is to validate one experimental method that could predict the results of *in vivo* experiments.

At the recent WHO Workshop in Copenhagen (May 1992), it was stated that, for the assessment of the health risk involved with a fiber, an RCC-type animal inhalation test with rats should be used. The same type of animal inhalation test should also be used to evaluate the validity of a predictive *in vitro* method. To the extent that the results of an *in vitro* test correlate with the RCC-type animal test, the parameters chosen will have been shown to be correct and adequate.

Six man-made mineral fibers (four refractory fibers and two glass fibers) of approximately the same geometry and known chemistry have been tested completely with an RCC-type animal test, and the percentage of tumors and the pulmonary reactions produced in the rats will soon be published. When they are available, they should be compared with the results of the *in vitro* measurement methods described, even if some of the methods are too limited and are not applicable to all six types of fibers tested in RCC.

An important ongoing experiment is comparing different *in vitro* methods using four fibers (MMVF11, MMVF10, MMVF21, MMVF22) (*21*). Once the tumor rates from the RCC-type experiments are available for comparison with this *in vitro* study, it will be possible to see how close is the correlation, and therefore which *in vitro* method should be recommended. However, for the moment only the dissolution rates *in vitro* and *in vivo* can be compared (Figure 4).

In summary, it would appear that the best *in vitro* test method would use Gamble's solution at pH 7.5, with fiber samples of the TIMA type, that is, with diameter approximately 1 μm and mean length approximately 20 μm. The test method should be dynamic, and the solubility determined by measuring the amounts of all components going into solution. The flow rate should be adjusted according to the solubility of the fiber, and should aim to keep the kinetic parameter constant.

In choosing the most suitable parameters for an *in vitro* test, it should be borne in mind that organic fibers, not inorganic, represent by far the largest part of all fibers present in indoor air. In the future, therefore, it may be necessary to develop a standard method for testing their biodurability.

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